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EDITORIAL

Why should an angiogenic factor modulate tubular structure in diabetic nephropathy? Some answers, more questions

In the late 1980s, the first two angiogenic growth factors, which were then thought to be highly specific for the vasculature, were isolated on the basis of their ability to mediate vascular leakage (vascular permeability factor) and proliferation of endothelial cells [vascular endothelial growth factor (VEGF)] [1]. It was found by nucleotide sequencing that these proteins were identical. The most widely studied member of the VEGF family, VEGF-A, is a 34–46 kD homodimeric glycoprotein that exists in at least five different splice isoforms [1]. In humans, $VEGF_{165}$ is the most abundantly expressed and secreted isoform. VEGF binds on target cells to at least two high-affinity tyrosine kinase receptors, VEGFR-1 and VEGFR-2 [1]. In the normal kidney, VEGFRs are expressed on endothelial, mesangial, and interstitial cells [2], while VEGF itself is expressed in podocytes, distal tubules, and collecting ducts [3]. An in vitro study demonstrated VEGF production in cultured human proximal tubules [4].

VEGF is a critical factor in the pathogenesis of proliferative diabetic retinopathy, but what is the rationale to study the VEGF system in the kidney? Since VEGF was originally characterized as a vascular permeability factor, it has been consequently implicated in several proteinuric diseases, including diabetic nephropathy. Circulating VEGF is elevated in diabetes, and increased renal VEGF mRNA and protein levels are found in early diabetic nephropathy [2]. In contrast, decreased VEGF content, probably due to podocyte loss, has been described in advanced diabetic glomerulosclerosis. In cultured mouse podocytes, high ambient glucose stimulates VEGF protein expression and this effect is largely mediated by the transforming growth factor-β (TGF-β) [5].

A functional role for VEGF in diabetic nephropathy was first demonstrated by the observation that monoclonal anti-VEGF antibodies administered to streptozotocin-diabetic rats decreased hyperfiltration, albuminuria, and glomerular hypertrophy [6]. Since $VEGF_{165}$ phosphorylates and activates eNOS, resulting in a local increase in nitric oxide (NO), prevention of NO formation could therefore explain how anti-VEGF antibody prevents diabetic hyperfiltration. A recent long-term study of anti-VEGF antibodies in *db/db* mice with type 2 diabetes showed significant attenuation of albuminuria, glomerular lesions, and kidney hypertrophy [7]. However, the role of VEGF in the etiology of proteinuria is not as straightforward as it seems. The cellular and molecular targets for the effects of VEGF on macromolecular permeability are far from being elucidated. On the other hand, neutralizing circulating VEGF in normal mice can actually induce proteinuria, and this is associated with down-regulation of nephrin expression [8]. In addition, exogenous $VEGF_{165}$ significantly enhances capillary repair and convincingly improves renal function in rats with experimental glomerulonephritis [3]. These data indicate that a certain amount of VEGF is necessary to maintain normal glomerular structure and function. Disturbances of this delicate balance by either under- or overexpression of VEGF may modulate the function of the filtration barrier.

Although such a complex role for an angiogenic factor in the glomerular microcirculation may be appreciated even by the skeptics, the study by Senthil et al [9] in this issue of *Kidney International* on VEGF's modulation of tubular epithelial structure in diabetes comes somewhat as a surprise. Accumulating evidence suggests that disruption of the tubulointerstitial architecture determines the outcome of diabetic nephropathy [10]. In fact, proximal tubular cell growth is one of the earliest renal abnormalities detected in diabetes [10]. It has been proposed that high glucose–induced tubular hypertrophy results in increased proximal reabsorption, which is sufficient to reduce the signal for tubuloglomerular feedback, thereby causing the glomerular filtration rate to increase [11]. On the other hand, there is increasing evidence that this initial tubular hypertrophy evolves into a maladaptative process through various mechanisms, including generation of reactive oxygen species, secretion of proinflammatory cytokines, expression of potential autoantigens, and probably transdifferentiation into collagen-secreting fibroblasts [10]. The morphologic end points of this process are tubular atrophy and interstitial fibrosis.

Senthil et al [9] now report an early increase in $VEGF_{165}$ mRNA and protein expression in renal cortices of mice with type 1 and type 2 diabetes [9]. The increase in VEGF expression coincides with the development of kidney hypertrophy. However, the exact cell type responsible for VEGF up-regulation was not determined, but it is reasonable to assume that the tubular compartment significantly contributed to this increase. The au-

thors then tested whether exogenous VEGF may activate signal transduction pathways in a cultured mouse proximal tubular cell line (MCT cells). Western blotting revealed the presence of VEGFR-2 but not VEGFR-1 in these cells. Exogenous VEGF resulted in tyrosine phosphorylation of VEGFR-2. VEGF stimulated PI 3-kinase and also activated its downstream target, Akt/PKB. This treatment was associated with increased de novo protein synthesis. Inhibition of VEGF-mediated PI 3-kinase and Akt activation abolished VEGF-mediated protein synthesis. Finally, VEGF stimulated phosphorylation of eukaryotic initiation factor 4E binding protein (4E-BP1) in an Akt-dependent manner, indicating that early events in protein translation may explain the stimulation in protein synthesis.

Like any novel observation, the present study provides raw material for further investigations. It would be important to clarify the nonangiogenic or hypertrophic effects of VEGF on the kidney in vivo. Although Senthil et al [9] have shown that VEGF stimulates de novo protein synthesis in cultured proximal tubular cells, this may not definitively prove that tubuloepithelial hypertrophy was actually induced by VEGF. An increase in protein synthesis is observed during different phases of the cell cycle and also occurs during cellular proliferation. Criteria to measure hypertrophy have been clearly defined and involve also looking at cell cycle regulation [10]. Since potential proliferative effects were not assessed, it remains uncertain whether VEGF really induces hypertrophy. Nevertheless, the observation that anti-VEGF antibody can reduce the increase in kidney weight in diabetic *db/db* mice [7] support some role for VEGF in tubuloepithelial hypetrophy. Finally, the relationship of VEGF to other growth factors needs to be clarified. It has been previously demonstrated in vivo and in vitro that interference with TGF-β activation completely abolishes tubular hypertrophy [10]. Since TGF-β induces VEGF synthesis at least in podocytes [5], it would be interesting to study whether some of the established effects of TGF-β in diabetic nephropathy are actually mediated through VEGF.

In summary, the present study by Senthil et al opens new avenues of research in the pathophysiology of tubular hypertrophy in diabetes and the emerging role of VEGF in the structural and functional manifestations of diabetic nephropathy.

Fuad N. Ziyadeh and Gunter Wolf
Philadelphia, Pennsylvania, and Hamburg, Germany

Correspondence to Fuad N. Ziyadeh, M.D., Renal-electrolyte and Hypertension Division, 700 Clinical Research Building, University of Pennsylvania, 415 Curie Boulevard, Philadelphia, PA 19104.
E-mail: ziyadeh@mail.med.upenn.edu

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